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Morpho-phylogenetic evidence for the first freshwater record of *Trichoderma protrudens* Samuels & P. Chaverri (Hypocreales, Sordariomycetes)

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Abstract. *Trichoderma protrudens* Samuels & P. Chaverri was originally described from the trunk of *Theobroma cacao* L. in Kerala, India, in 2008. Morphologically, it is defined by trichoderma-like conidiophores bearing subulate or lageniform phialides, green subglobose to obovoidal conidia, and the presence of chlamydospores in culture. Multilocus phylogenetic analyses based on ITS, *rpb2*, and *tef1-α* sequence data confirmed the identity of the isolates from the Philippines as *T. protrudens*, with robust support values (100% ML, 1.00 BPP) clustering them with the holotype CBS 121320. This study represents the first global record of *T. protrudens* in a freshwater ecosystem, expanding this species' known ecological range into aquatic environments. These findings emphasize the ecological versatility of *T. protrudens* and underscore the importance of further investigations into the fungal diversity of freshwater habitats.

Key words. Biodiversity, fungal systematics, microbial taxonomy, mycology, Philippines, tropical mycology

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INTRODUCTION

Lignicolous freshwater fungi, an important ecological group, thrive in freshwater habitats by utilizing submerged decaying plant matter (Jones et al. 2014). These fungi are vitally important in freshwater ecosystems in that they decompose lignocellulose and contribute to the carbon and nutrient cycles (Wong et al. 1998; Bucher et al. 2004; Hyde et al. 2016). Since 1996, extensive research has led to the description of over 3,870 freshwater fungal species, primarily ascomycetes (Calabon et al. 2022; Goh and Hyde 1996). The discovery of new taxa continues to accelerate, with no signs of slowing down (Calabon et al. 2023).

Trichoderma Pers. (Hypocreales) was first described by Pearson with *T. viride* Pers. as the type species (Pearsoon 1794). It is the most diverse member of the Hypocreales, with a global distribution and an estimated 400+ species (Sadfi-Zouaoui et al. 2009; Jaklitsch and Voglmayr 2015; Ma et al. 2020; Wijayawardene et al. 2020; Cai and Druzhinina 2021; Cai et al. 2022; Tang et al. 2022). The diversity and interest in *Trichoderma* stem from its production of numerous secondary metabolites with ecological and economic significance (Khan et al. 2020a). In agriculture, *Trichoderma* inoculants have been used for pest and disease control, and as growth promoters (Khan et al. 2020b; Zin and Badaluddin 2020; Subramaniam et al. 2022; Tyśkiewicz et al. 2022; Yao et al. 2023; Kredics et al. 2024). Additionally, some *Trichoderma* species exhibit stress tolerance and can absorb heavy metals and organic pollutants, making them potential agents for environmental remediation (Bishnoi et al. 2007; Zafra and Cortés-Espinosa 2015; Cuevas and Banaay 2022).

In freshwater ecosystems, *Trichoderma* species play a crucial ecological role through their lignocellulolytic activity, which allows them to colonize and degrade lignocellulosic substrates, even in submerged aquatic habitats (Savory 1954; Calabon et al. 2023; Huang et al. 2024). The presence of saprobic *Trichoderma* in freshwater environments has been well-documented across several regions. For instance, studies by Au et al. (1992), Pinruan et al. (2014), Goh et al. (2018), and Concha et al. (2024) reported their occurrence on various submerged decaying plant substrates and sediments in China, Korea, Thailand, and the Philippines. Zheng et al. (2021) expanded the understanding of *Trichoderma* diversity in aquatic habitats by describing eight endophytic species associated with freshwater plants in China. These findings underscore the adaptability and functional importance of *Trichoderma* in freshwater ecosystems, particularly in processes involving organic matter decomposition and nutrient cycling.

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Traditionally, *Trichoderma* species have been identified and classified based on morphological analysis and growth rates (Bissett 1984, 1991a, 1991b; Harman and Kubicek 2002; Siddiquee 2017). However, morphological traits can be homoplastic, leading to challenges in identification (Druzhinina et al. 2005, 2006). DNA barcoding, using internal transcribed spacer (ITS), translation elongation factor 1-alpha (*tef1-α*), and RNA polymerase II gene (*rpb2*), has become a widely used method for *Trichoderma* and other hypocrealean fungi (Kuhls et al. 1996; Kullnig-Gradinger et al. 2002; Chaverri et al. 2015; Inglis et al. 2020; Cai and Druzhinina 2021; Cai et al. 2022). The simultaneous use of *tef1-α* and *rpb2* for phylogenetic analyses is particularly effective in identifying novel *Trichoderma* species, as these protein-coding genes offer greater resolution than ribosomal genes. (Hanada et al. 2008; Stielow et al. 2015; Zhu and Zhuang 2015; Qin and Zhuang 2016; Ye et al. 2023). The concatenation of multiple loci has been widely adopted to enhance resolution and accuracy in phylogenetic analyses, particularly in the reconstruction of fungal phylogenies (Delsuc et al 2005; Robbertse et al. 2006; Marcet-Houben and Gabaldón 2009; Cai and Druzhinina 2021). A standardized protocol has been developed, requiring a *tef1-α* similarity of ≥97% and an *rpb2* similarity of ≥99% for species identification. Failure to meet these criteria suggests the potential for a novel species (Cai and Druzhinina 2021).

Aquatic *Trichoderma* species have been previously isolated from submerged substrates like sand sponges and decaying wood in marine environments (Paz et al. 2010; Gal-Hemed et al. 2011; Goh et al. 2018; Zheng et al. 2021). While freshwater *Trichoderma* has not been extensively surveyed in the Philippines, it has found application in agriculture as a sustainable alternative to synthetic fertilizers and fungicides (Cuevas et al. 2005; Launio et al. 2020). In this study, *Trichoderma* strains were isolated from submerged wood and bamboo substrates collected from a freshwater habitat in Iloilo, Philippines. This discovery was based on a comprehensive morphological and multilocus phylogenetic analysis, highlighting the diversity and potential of *Trichoderma* in freshwater ecosystems.

METHODS

Wood samples were collected from a waterfall in Miagao, Iloilo, Philippines (Figures 1, 2). These specimens were examined using the methods outlined by Senanayake et al. (2020). The incubated wood samples were initially observed under a stereomicroscope. The morphology of the observed fruiting bodies on the wood substrate was photographed using a Kuaiqu Simul-Focal Trinocular dissecting microscope. Microscopic characters were captured using an Olympus BX53 microscope. The microphotographs were then processed using Adobe Photoshop(v. 21.1 and important characters were measured with ImageJ v. 154j. Single-spore isolation was conducted using potato dextrose agar (PDA) plates, following the procedures described by Senanayake et al. (2020). Mycelial growth patterns were observed on PDA, malt extract agar (MEA), and cornmeal agar (CMA) after seven and fourteen days. Fungal herbarium specimens and pure living cultures were stored and deposited in the University of the Philippines Visayas–Division of Biological Sciences Herbarium (UPVMI) and Culture Collection (UPVMICC) (Accessed on 2025-9-15), respectively.

Fresh fungal mycelia were scraped from colonies on PDA plates and placed in sterile 1.5 mL microcentrifuge tubes. Genomic DNA was extracted using QIAGEN DNeasy extraction kits following the manufacturer's protocol. Polymerase chain reaction (PCR) was used to amplify the ITS, *tef1-α*, and *rpb2* markers. The ITS markers were amplified using ITS5 and ITS4 primers (White et al. 1990). *tef1-α* and *rpb2* were amplified using EF1-983f and EF1-2218R (Rehner and Buckley 2005) and fRPB2–5f and fRPB2–7cr (Liu et al. 1999), respectively. Each 25 µL PCR reaction consisted of 18.37 µL nuclease-free water, 2.5 µL genomic DNA, 2.5 µL PCR buffer solution, 0.5 µL dNTPs, 0.5 µL forward and reverse primers, and 0.125 µL HotStartaq DNA polymerase. The PCR program began with an initial denaturation at 95 °C for 15 min, followed by 35 cycles of denaturation

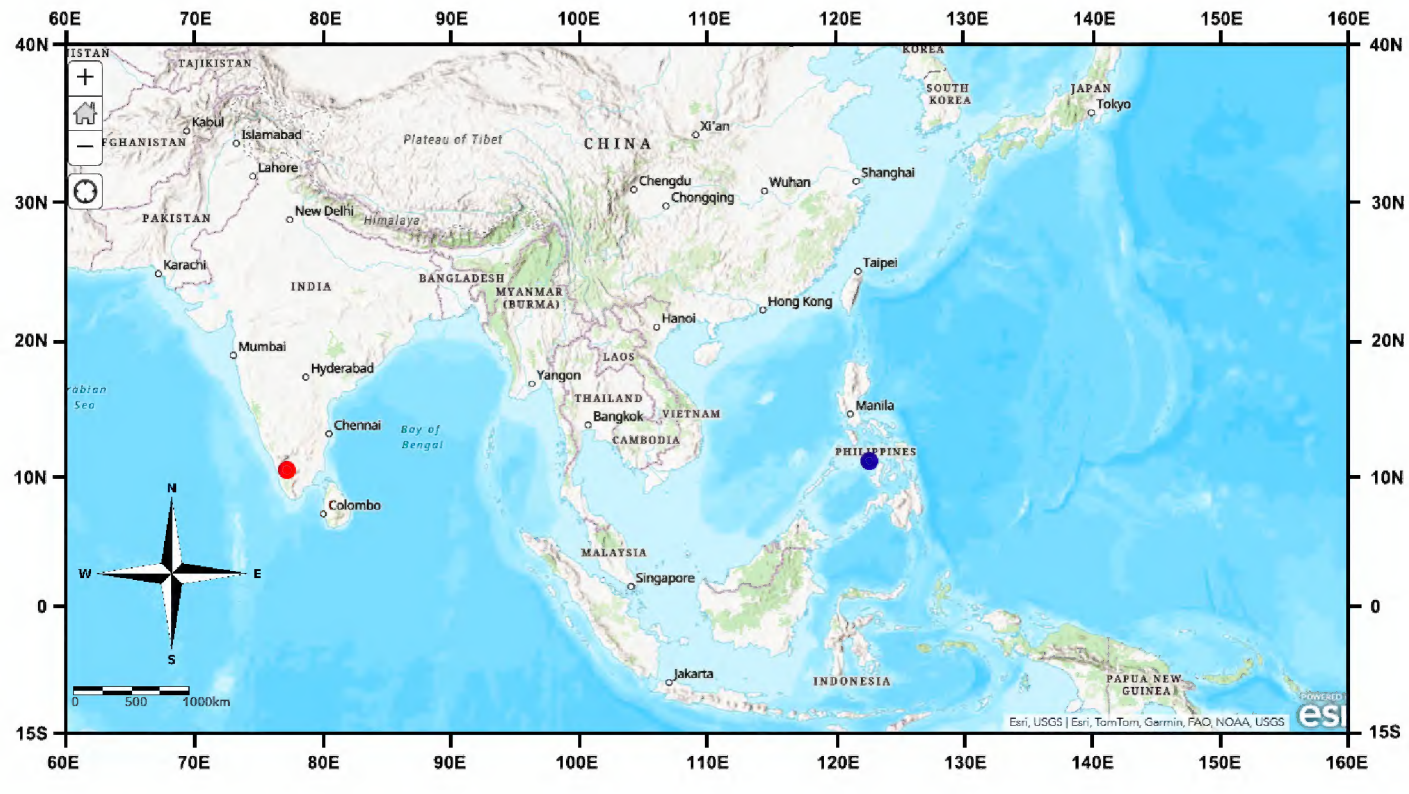

Figure 1. Location map showing the collection site of *Trichoderma protrudens*. The blue and red circles denote the new record in the Philippines (this study) and India (Degenkolb et al. 2008), respectively. The map was created using ArcGIS.

Figure 2. Habitat and collection of *Trichoderma protrudens* in the Philippines. **A.** Dilaan Falls, Miagao, Iloilo. **B, C.** Collection and sorting of samples. **D, E.** Unidentified submerged decaying woods: (**D**) UPVMI 24-0008 and (**E**) UPVMI 24.0009 (arrows point to colonies of *T. protrudens* on the natural substrate).

(1 min at 95 °C), annealing (30 s at 56 °C), and elongation (1 min at 72 °C). A final extension step was performed at 72 °C for 10 min. The presence of amplicons was confirmed using agarose gel electrophoresis on a Mupid One Electrophoresis System. The PCR products were sequenced on the Illumina iSeq100 platform at the Philippine Genome Center Visayas, University of the Philippines Visayas, Miagao, Philippines.

The taxa included in our phylogenetic analysis (Table 1) were assembled based on the closest matches from the BLAST search results and from recently published data of Brito et al. (2023). Sequences generated for the three target genes were combined with additional sequences retrieved from GenBank (Table 1). Three separate datasets, one for each gene, were aligned using MAFFT v. 7 (Katoh et al. 2019). Alignments were then manually refined where necessary with BioEdit v. 7.0.9.0 (Hall 1999). TrimAl v. 1.3 was employed for automatic trimming of aligned sequences. The online tool ALTER (Glez-Peña et al. 2010) facilitated conversion of the alignment file to Phylip format. Both individual gene data and combined data were subjected to phylogenetic analysis using the maximum-likelihood (ML) method. RAxML v. 8 software on the CIPRES web portal (Stamatakis 2014) (http://www.phylo.org/portal2/; Miller et al. 2010) was utilized for this purpose. The GTR+GAMMA model of nucleotide evolution was adopted, and RAxML rapid bootstrapping was performed with 1,000 replicates. Additionally, Bayesian-inference analysis was conducted with MrBayes v. 3.2.6 on XSEDE at the CIPRES web portal (Ronquist and Huelsenbeck 2003). Newly generated sequences were deposited in GenBank (Table 1).

Table 1. Taxa used in this study for the analysis of combined ITS rDNA, *rpb2*, and *tef1-α* sequence data and their GenBank accession numbers. The newly generated sequences are indicated with asterisk (*).

Taxon	Strain / voucher number	ITS	rpb2	*tef1-α*
Protocrea farinosa	CBS 121551	MH863119	EU703935	EU703889
P. pallida	CBS 121552	EU703922	EU703944	EU703897
Trichoderma albolutescens	CBS 119286	FJ860721	FJ860517	FJ860609
T. applanatum	7792	KJ783291	KJ634726	KJ634759
T. arundinaceum	37MTS	OR880618	–	PP263665
	GJS 05-184	EU330933	EU338308	EU338280
	NRRL 3199	EU330932	EU338307	EU338279
T. atlanticum	CBS 120632	FJ860781	FJ860546	FJ860649
T. atroviride	CBS:142.95	AF456917	EU341801	AF456891
T. aurantioeffusum	CBS 119284	FJ860728	FJ860520	FJ860613
	CPK 3119	FJ860730	FJ860521	FJ860614
	S565	–	–	KJ665430
T. austriacum	CBS 122494	FJ860735	FJ860525	FJ860619
T. brasiliensis	COAD 2324	MK713497	MT300488	MT300486

Taxon	Strain / voucher number	ITS	rpb2	*tef1-α*
	COAD 2642	MK713498	MT300490	MT300487
	COAD 2643	MK713499	MT300489	MT300492
	COAD 2644	MK713514	MT300491	MT300493
T. brevicompactum	CBS 109720	AY857249	DQ857347	AY324176
	CBS 112443	EU330943	EU338319	EU338281
	CBS 112444	EU330938	EU338314	EU338296
	CBS 112447	EU330942	EU338318	EU338300
T. citrinum	CBS 894.85	MH861923	AF545561	DQ835441
T. grande	HMAS:248749	–	KX066266	KX066254
	HMAS:273788	–	–	KX066255
T. limonium	HMAS:248751	–	KX066259	KX066247
	HMAS:248754	–	KX066260	KX066248
T. luteoeffusum	CBS 120537	FJ860773	FJ860543	FJ860645
T. margaretense	C.P.K. 3127	FJ860741	FJ860529	FJ860625
T. oligosporum	HMAS:252870	KJ783296	KJ634731	KJ634764
T. peltatum	GJS 08-207	HM466659	HQ260610	KR135819
T. phellinicola	CBS 119283	FJ860808	FJ860569	FJ860672
T. polysporum	CBS:820.68	MH859230	DQ087238	AF400989
T. protopulvinatum	CBS 739.83	DQ835405	DQ835463	DQ835487
T. protrudens	CBS 121320	EU330946	EU338322	EU338289
	UPVMICC 24-0008 *	PQ451471	PQ790167	PQ790164
	UPVMICC 24-0009 *	PQ451472	–	PQ790165
T. pseudostramineum	TUFC60104	JQ797392	JQ797408	JQ797400
T. psychrophilum	C.P.K. 2435	FJ860819	FJ860576	FJ860682
T. pulvinatum	CBS 121279	FJ860820	FJ860577	FJ860683
T. rhododendri	CBS 119288	FJ860822	FJ860578	FJ860685
T. rodmanii	GJS 91-88	EU330948	EU338324	EU338286
	CBS:121553	FJ860824	FJ860580	FJ860687
	CPK 2852	FJ860825	FJ860581	FJ860688
T. seppoi	CBS:122498	FJ860833	FJ179617	FJ179581
T. stercorarium	CBS:148.85	MH861866	EF469103	AF543782
T. subsulphureum	M 141	DQ835509	DQ835522	DQ835492
T. tiantangzhaiense	HMAS 252872	KJ783295	KJ634730	KJ634763
T. turrialbense	CBS 112445	EU330945	EU338321	EU338284
	BBA 72294	EU330944	EU338320	EU338282
	SFC20160125-C300	–	–	MZ634448
T. undulatum	PPRI:19365	KX267810	KX351319	KX267789
T. victoriense	CBS:140064	DQ835505	DQ835517	DQ835500
T. viride	CBS:119325	DQ323428	EU711362	DQ672615

RESULTS

Trichoderma protrudens **Samuels & P. Chaverri**, Mycol. Progr. 7(3): 212 (2008)
Figure 3

New record. PHILIPPINES – Iloilo • Miagao, Dilaan Falls; 10°44′37.98″N, 122°11′22.0″E; 23.IX.2023; B.S. Dangallo leg.; isolated from submerged decaying unidentified wood; tropical monsoon climate; GenBank ITS rDNA PQ451471; *tef1-α* PQ790164; *rpb2* PQ790167; UPVMI 24-0008; living culture UPVMICC 24-0008; GenBank ITS rDNA PQ451472; *tef1-α* PQ790165; UPVMI 24-0009; living culture UPVMICC 24-0009.

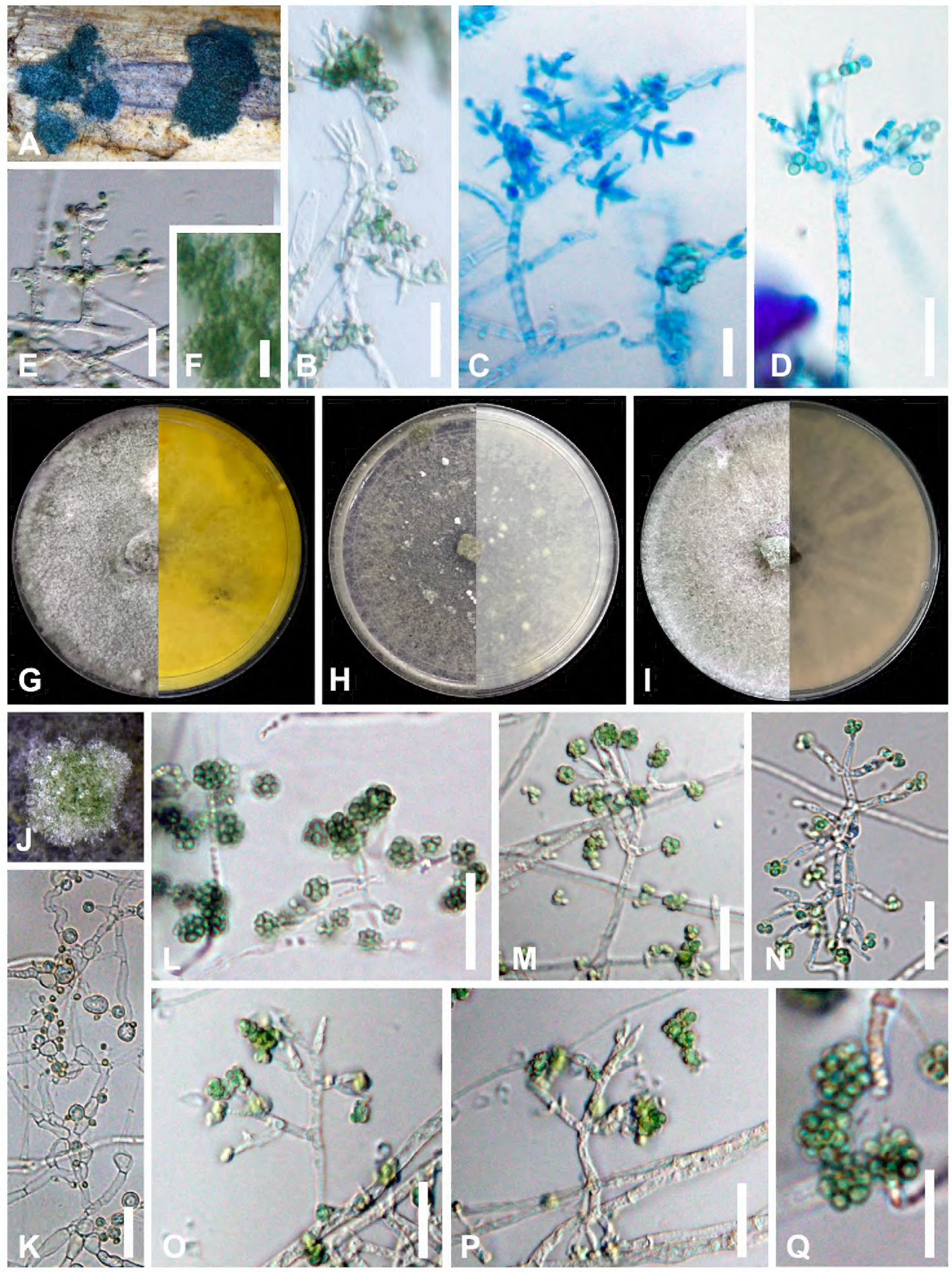

Figure 3. *Trichoderma protrudens* (24-0008) **A.** Appearance of fungi on bamboo. **B–E.** Conidiophore and phialides: (**B-D**) with and (**E**) without lactophenol cotton blue stain. **F.** Conidia. **G–I.** Colonies after 14 days: (**G**) on potato dextrose agar; (**H**) on corn meal agar; (**I**) on malt extract agar. **J.** Active sporulation on CMA. **K.** Chlamydospores. **L–Q.** Conidiophores and phialides with conidia clusters. Scale bars: B–E, K, L–P = 20 µm; F, Q = 10 µm.

Identification. *Saprobic* on decaying wood submerged in freshwater. *Description on natural substrate*: sexual morph undetermined. Asexual morph: hyphomycetous. Conidial pustules, dark bluish green, irregular shape, outline not well defined. Conidiophores 35–155 × 1.9–4.1 µm ($\bar{x}$ = 67.1 × 2.9 µm, *n* = 30), trichoderma-like, hyaline, often symmetry, repeatedly branched toward the base forming a pyramidal structure with phialides in whorl, smooth-walled, often with a main axis, side branches in straight angles with main axis. Phialides 10–18 µm length ($\bar{x}$ = 14.2 µm, *n* = 50), 1.9–3.8 µm maximum width ($\bar{x}$ = 2.8 µm), 1.7–2.7 µm base width ($\bar{x}$ = 2.3 µm), typically formed in whorls of 3 or rarely solitary, subulate or lageniform. Conidia 3.5–5.0 × 1.9–2.9 µm ($\bar{x}$ = 4.1 × 2.5 µm, *n* = 50), green, smooth, subglobose to obovoidal.

Culture characteristics: on MEA: after 72 h, colony radius reached 33–38 mm at 25 °C, colonies on MEA reached 90 mm diameter within 5 days at 25 °C; Colony radial, mycelium common, aerial hyphae more abundant in colony margin. Conidiation starting after 6 days, light-green conidia formed scarcely on aerial hyphae: few pustules. On PDA: after 72 h, colony radius reached 32–36 mm at 25 °C. Conidiation starting after eight days, light-green conidia formed scarcely on aerial hyphae, more abundant around the margin of the colon; few pustules. No pigment diffusing through the agar; no distinctive odor. On CMA: after 72 h, colony radius reached 28–31 mm at 25 °C, colonies reached 90 mm diameter at four days and 25°C; cottony white aerial mycelium, light-green sporulation starting from the center of the plate, pustules abundant. Conidiation starting after 5 days, light-green conidia formed on aerial hyphae around margin of colony. No pigment diffusing through the agar; no distinctive odor. Conidiophores trichoderma-like, micronematous, pyramidal with phia-

lides in whorl, smooth-walled. Phialides 9–27 µm length ($\bar{x}$ = 16, *n* = 50), 2–5 µm maximum width ($\bar{x}$ = 3 µm), 1.8–4.4 µm base width ($\bar{x}$ = 2.7 µm), hyaline, typically formed in whorls of 3 or rarely solitary, subulate or lageniform. Conidia 1.6–3.3 × 1.9–2.9 µm ($\bar{x}$ = 2.2 × 2.1 µm, *n* = 50), pale greenish, smooth, subglobose to obovoidal. Chlamydospores 2.4–9.2 × 2.6–9.7 ($\bar{x}$ = 6.6 × 5.9 µm, *n* = 30), hyaline, abundant, globose.

In a multilocus phylogenetic analysis, strains UPVMICC 24-0008 and UPVMICC 24-0009 clustered with the holotype CBS 121320 with strong statistical support (100% ML, 1.00 BPP) (Figure 4). The single-locus trees revealed that these strains fall within the brevicompactum clade (Figures 5, 6). In the *rpb2* phylogenetic tree, strain UPVMICC 24-0008 clustered with the holotype CBS 121320 with strong statistical support (100% ML) (Figure 5). However, in the *tef1-a* locus, both UPVMICC 24-0008 and UPVMICC 24-0009 exhibited a distinct lineage basal to *T. protrudens* (Figure 6). This unique lineage in the *tef1-a* tree could be attributed to the use of primers EF1-983f and EF1-2218R, as opposed to the standard *tef1-a* barcodes (EF1 and EF2) recommended by Cai and Druzhinina (2021). Morphologically, *T. protrudens* UPVMICC 24-0008 and UPVMICC 24-0009 have trichoderma-like conidiophore with smaller conidia [1.6–3.3 × 1.9–2.9 µm vs. (2.5–)2.7–3.2(–3.5) × (2.2–)2.5–2.7(–3.0)], and develops chlamydospores in the culture medium, features that distinguish the isolate from the holotype CBS 121320 (Degenkolb et al. 2008). Based on morphological characteristics and phylogenetic analyses of ITS, *rpb2*, and *tef1-α* regions, these two isolates have been identified as *T. protrudens*, marking the first report of this species in a freshwater ecosystem globally.

Habitat. Trunk of *Theobroma cacao* (Degenkolb et al. 2008); on decaying wood submerged in a freshwater stream (this study).

Known distribution. India (Degenkolb et al. 2008), Philippines (this study).

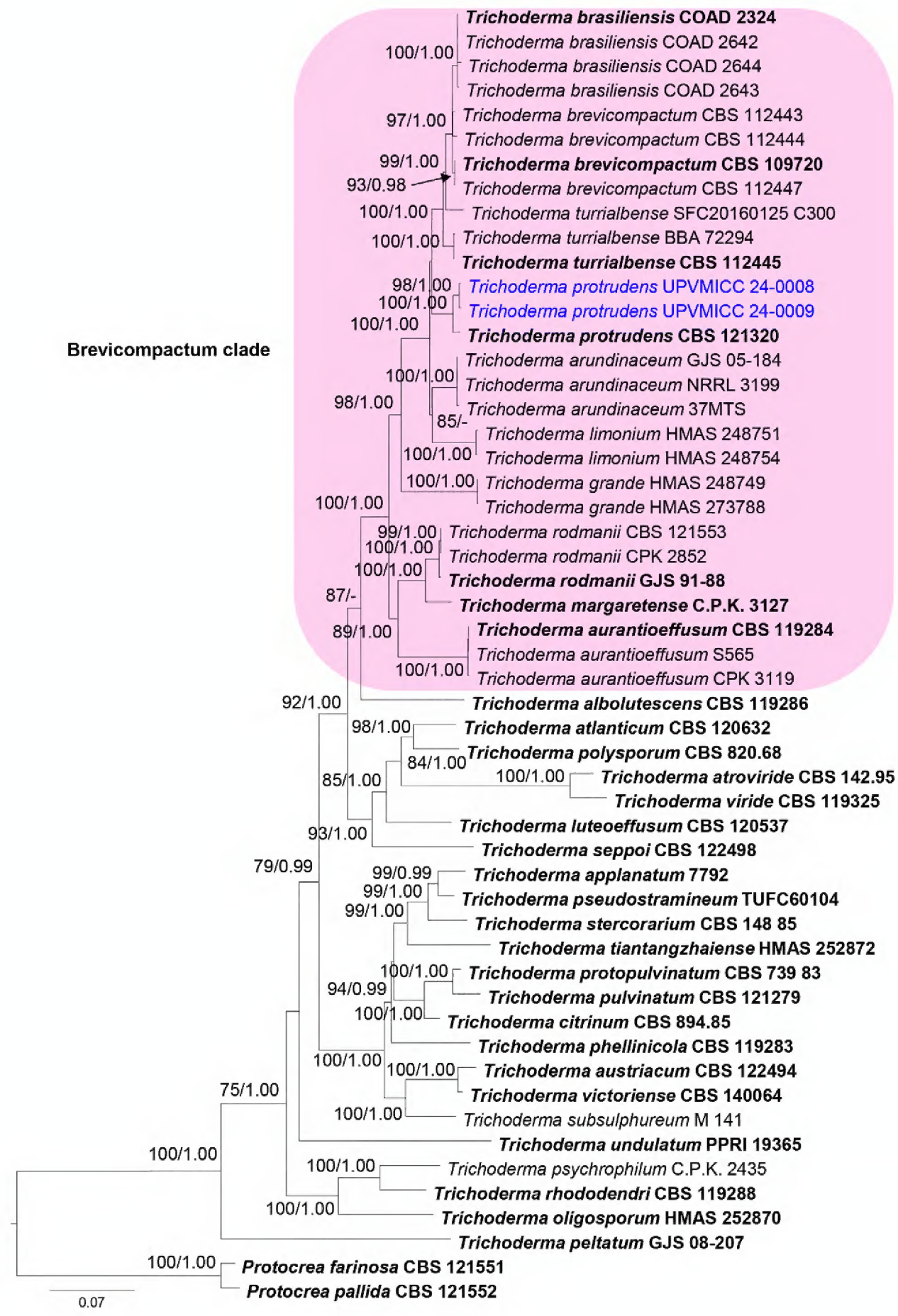

Figure 4. Phylogenetic tree generated from maximum-likelihood (ML) analysis based on ITS, *rpb2*, and *tef1-α* sequence data for the species of *Trichoderma*. Bootstrap support values for maximum likelihood (ML) higher than 75% and Bayesian posterior probabilities (BPP) greater than 0.95 are indicated above the nodes in this order. The new isolates are represented in blue. The ex-type strains are indicated in bold. The tree is rooted to *Protocrea farinosa* (CBS 121551) and *P. pallida* (CBS 121552). Scale bar = 0.07 estimated number of nucleotide substitutions per site per branch.

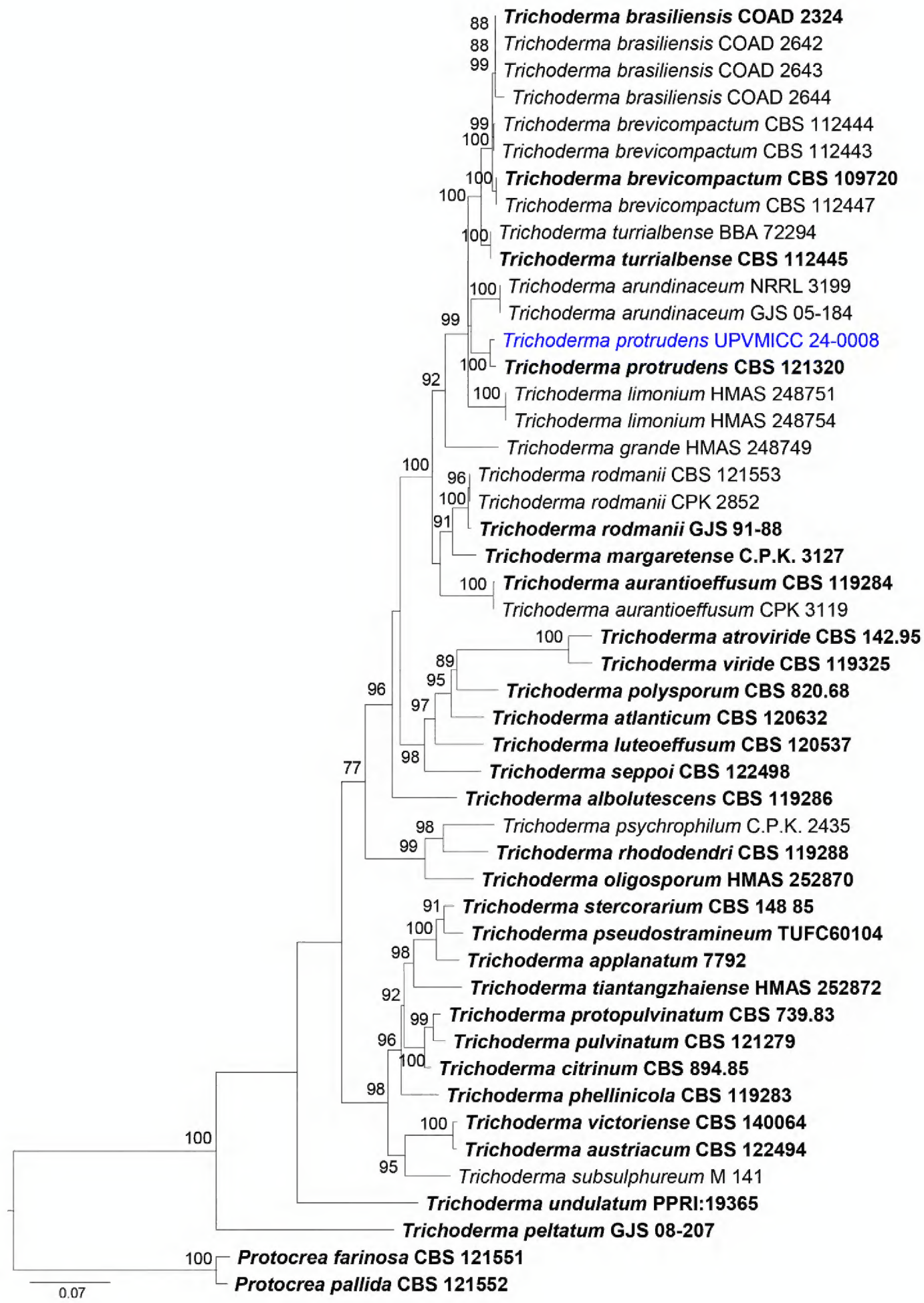

Figure 5. Phylogenetic tree generated from maximum-likelihood (ML) analysis based on *rpb2* sequence data for the species of *Trichoderma*. Bootstrap support values for maximum likelihood (ML) higher than 75% and Bayesian posterior probabilities (BPP) greater than 0.95 are indicated above the nodes in this order. The new isolates are represented in blue. The ex-type strains are indicated in bold. The tree is rooted to *Protocrea farinosa* (CBS 121551) and *P. pallida* (CBS 121552). Scale bar = 0.07 estimated number of nucleotide substitutions per site per branch.

DISCUSSION

Based on a multilocus phylogenetic analysis of combined datasets of ITS, *rpb2*, and *tef1-α*, the newly isolated strains were placed within the brevicompactum clade. The brevicompactum clade, one of the infrageneric clades of *Trichoderma*, is distinguished by its relatively small number of species (only 14) and its characteristic morphological features, including globose to subglobose conidia and ampulliform to lageniform phialides. While some species within this clade have been observed to produce sexual morphs (e.g., *T. rodmanii* (Samuels & P. Chaverri) Jaklitsch & Voglmayr, *T. grande* W.T. Qin & W.Y. Zhuang, *T. limonium* W.T. Qin & W.Y. Zhuang), the majority are known only as asexual morphs (Degenkolb et al. 2008).

Aquatic Hypocreales encompass 80 genera across six families. Most of these genera (57) belong to the family Nectriaceae (Calabon et al. 2022). Over the past decade, there has been a surge in the discovery and description of novel freshwater Hypocreales species and genera. For instance, *Penicillifer sinicus* Z.Q. Zeng & W.Y. Zhuang, *Fusicolla aquaeductuum* (Radlk. & Rabenh.) Gräfenhan, Seifert & Schroers, and *Aquanectria jacinthicolor* S.K. Huang, Jeewon & K.D. Hyde were identified in China, while the genera *Varicosporella* Lechat & J. Fourn. and *Cosmosporella* S.K. Huang, Jeewon & K.D. Hyde were discovered in France and China, respectively (Lechat and Fournier, 2015; Huang et al. 2018; Zeng and Zhuang 2022). The adaptations of Hypocreales to freshwater habitats remain a subject of ongoing research. While there is no clear evidence of specialized

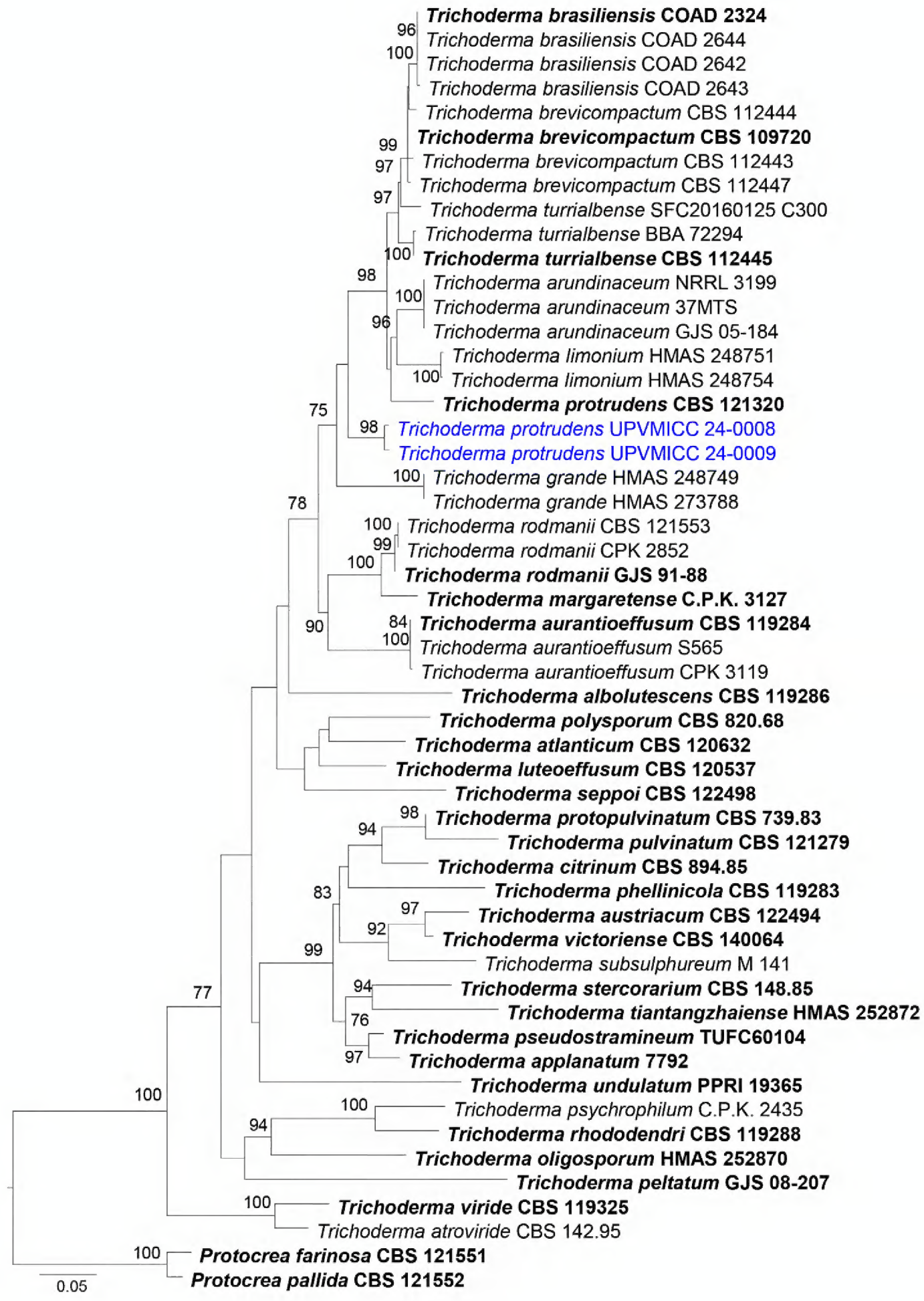

Figure 6. Phylogenetic tree generated from maximum-likelihood (ML) analysis based on *tef1-α* sequence data for the species of *Trichoderma*. Bootstrap support values for maximum likelihood (ML) higher than 75% and Bayesian posterior probabilities (BPP) greater than 0.95 are indicated above the nodes in this order. The new isolates are represented in blue. The ex-type strains are indicated in bold. The tree is rooted to *Protocrea farinosa* (CBS 121551) and *P. pallida* (CBS 121552). Scale bar = 0.05 estimated number of nucleotide substitutions per site per branch.

appendages, some species of Nectriaceae exhibit apical rings that may contribute to their aquatic lifestyle (Bao et al. 2023). The total number of *Trichoderma* species described is estimated to be between 400 and 600 (Sadfi-Zouaoui et al. 2009; Jaklitsch and Voglmayr 2015; Ma et al. 2020; Wijayawardene et al. 2020). According to *Index Fungorum* (as of 2024-10-10), there are 476 individual species recorded, excluding former species and duplicates. Recent studies have identified sixteen *Trichoderma* species in freshwater environments, where they function as saprobes and endophytes on various substrates across China, Korea, Philippines, and Thailand (Au et al. 1992; Pinruan et al. 2014; Goh et al. 2018; Zheng et al. 2021; Concha et al. 2024). A synopsis of freshwater *Trichoderma* species is provided in Table 2. These species are classified within six distinct *Trichoderma* species complexes: atroviride (*T. uncinatum*), hamatum (*T. pseudoasperelloides*), *harzianum* (T. *afroharzianum*, *T. guizhouense*, *T. harzianum*, *T. pinicola*), koningii (*T. tibetica*), longibrachiatum (*T. aquaticum*, *T. capillare*, *T. reesei*, *T. saturnisporum*), and viridescens (*T. asymmetricum*, *T. inconspicuum*, *T. paraviridescens*, *T. scorpioideum*). Despite this relatively small number in freshwater environments, the past decade has witnessed a significant increase in the discovery of *Trichoderma* species in terrestrial habitats, particularly in China (Qin and Zhuang 2016; Zheng et al. 2021; Ye et al. 2023; Zhao et al. 2023, 2024).

Taxonomic research on freshwater fungi in the Philippines remains underexplored, with most species' identification based primarily on morphological characteristics. Over the past two decades, only four species of freshwater fungi have been described: *Paoayensis lignicola* Cabanela, Jeewon & K.D. Hyde (Cabanela et al.

Table 2. Synopsis of morphological characters of freshwater *Trichoderma* species.

Taxon	Conidiophore		Phialides			Conidia				Chlamydospores		Reference
	Size (µm)	Shape	No.	Size (µm)	Shape	Size (µm)	Shape	Septa	Wall	Size (µm)	Shape	
T. afroharzianum	–	Pyramidal with opposing, widely spaced branches	1–5	3.5–17.5 × 2.0–4.2	Lageniform to ampulliform	2.0–4.5 × 2.0–4.0	Subglobose to ovoid	–	–	Rare		Chaverri et al. 2015
T. aquaticum	5.5–19.1	Pyramidal; alternately or irregularly branched	3–5	5.2–11.5 × 2.5–4.2	Lageniform, rare ampulliform, slightly curved	3.3–4.8 × 2.4–3.3	Mostly ovoid, ellipsoidal, rarely subglobose	–	Thin	4.3–7.6 × 3.91–7.2	Globose	Zheng et al. 2021
T. asymmetricum	–	Tree-like, asymmetrically branched	1–3	4.4–12.6 × 2.3–4.4	Occasionally lageniform, with obtuse apex	3.5–5.0 × 3.2–4.1	Subglobose to globose, sometimes broadly ellipsoidal, rarely ovoid	–	–	–	–	Zheng et al. 2021
T. capillare	–	Lateral branching with branches terminating in whorls of few phialides; sometimes, lacking branches lack obvious patterns	–	4.0–14.0 × 2.2–4.5	Lageniform, nearly cylindrical or conspicuously swollen below middle, straight or less frequently asymmetric or hooked	2.2–4.5 × 1.7–4.0	Subglobose to broadly ellipsoidal	–	–	Not observed		Samuels et al. 2012
T. guizhouense	–	Verticillate, forms a more or less pyramidal structure	2–3	4.5–10 × 2–3	Ampulliform to lageniform	2–3 (diam.)	Globose	–	Smooth	Not observed		Li et al. 2012
T. harzianum	–	Pyramidal with opposing branches	2–5	4.7–16 × 2.7–4.7	Ampulliform to lageniform	2.2–4.2 × 2.0–3.7	Subglobose to ovoid	–	–	Rare		Chaverri et al. 2015; Jang et al. 2018
T. inconspicuum	–	Tree-like to irregular branched	3–4	6.4–16.2 × 1.9–4.0	Narrow lageniform, some swollen in the middle, ampulliform	2.8–4.5 × 2.6–4.0	Subglobose to globose; few are ovoid	–	Thin	6.6–10.1 × 5.8–8.7	Globose, ellipsoidal	Zheng et al. 2021
T. paraviride	–	Curved to sinuous, branched irregularly or paired in tree fashion	3–5	9.2–16.4 × 2.1–4.1	Spindly to ellipsoid, rarely cylindrical, sometimes curved	3.4–5.0 × 3.2–4.8	Globose to subglobose	–	Thin	–	–	Zheng et al. 2021
T. paraviridescens	–	Tree/branch-like from, wide side branches	2–3	5.6–12.3 × 2.0–3.4	Lageniform	2.3–4.9 × 1.82–3.5	Globose to subglobose	–	–	6.08–11.17 (diam.)	Globose	Goh et al. 2018
T. pinicola	–	Pyramidal, branched repeatedly toward base	–	9–20 × 2–6	Subulate or lageniform	3.5–5.0 × 1.9–2.9	Oblong to ellipsoid	–	–	6.2–10 × 4–7	Globose	Concha et al. 2024
T. protrudens	–	broad central axis with lateral and often terminal fertile branches	–	–	Lageniform to ampulliform	(2.5–)2.7–3.2(–3.5) × (2.2–)2.5–2.7 (–3.0)	Subglobose to ovoidal	–	–	–	–	Degenkolb et al. 2008
T. *pseudoasperelloides*	–	Dendrifor, with little rebranching	3–5	5.0–9.0 × 2.0–5.0	Spindle-shaped and lageniform	3.7–4.1 × 2.7–3.8	Subglobose to ellipsoidal, rarely globose	–	Thin	–	–	Zheng et al. 2021
T. reesei	–	Long central axis with solitary phialides before secondary branching	–	3.0–11.5 × 1.8–4.0	Lageniform, asymmetric, swollen or not at middle, straight, less frequently sinuous	3.0–6.0 × 1.5–3.5	Ellipsoidal to oblong	–	–	–	Subglobose to pyriform	Goh et al. 2018
T. saturnisporum	–	Highly variable in form	–	5.5–14.0 × 2.0–3.5	Lageniform to ampulliform, often curved	3.0–6.0 × 2.0–4.0	Subglobose to ellipsoidal or subcylindrical	–	–	–	–	Goh et al. 2018
T. scorpioideum	–	Slightly curved main axis, generally verticillate branches	2–3	6.5–13.3 × 2.0–3.9	Narrow lageniform, some are ellipsoidal	3.3–4.4 × 2.4–3.8	Globose to subglobose, a few ovoidal to ellipsoidal	–	Thin	–	–	Zheng et al. 2021
T. tibetica	–	Slightly curved main axis, slightly upward and sometimes perpendicular branches	2–4	5.2–12.3 × 2.1–3.5	Lageniform, sometimes globose, ellipsoidal, or pyramidal	3.7–4.8 × 2.9–4.0	Ellipsoidal to ovoid, subglobose rarely noted	–	–	–	–	Zheng et al. 2021
T. uncinatum	–	Hard-discernable, slightly curved main axis, irregular alternate branches	2–5	4.3–10.3 × 2.3–3.9	Lageniform, sometimes ampulliform to subglobose	3.1–4.4 × 2.7–4.0	Globose to subglobose, rarely ovoidal or ellipsoidal	–	Thin	–	–	Zheng et al. 2021

2007), *Phaeoacremonium philippinense* M.S. Calabon, Bagacay, Nim, Sadaba & E.B.G. Jones (Calabon et al. 2024), *Sporoschisma chiangraiense* N.G. Liu & K.D. Hyde, and *Trichoderma pinicola* Seung Y. Oh, M.S. Park & Y.W. Lim (Concha et al. 2024), that employed a phylogenetic analysis of combined nuclear ribosomal DNA loci (LSU, SSU, ITS) and protein-coding genes (*tub2*, *act*, *tef1-α*). The Philippines remains underexplored in terms of freshwater fungal systematics research, especially when compared to nearby Asian countries such as China and Thailand (Zhang et al. 2011; Hu et al. 2013; Calabon et al. 2022). This underscores the vast yet largely untapped fungal diversity present in Philippine freshwater ecosystems. Comprehensive taxonomic surveys are essential to bridge this knowledge gap, integrating both morphological and molecular phylogenetic approaches to better characterize the region's fungal biodiversity. Globally, the occurrence and ecological roles of

Trichoderma in freshwater ecosystems are also underrepresented in scientific literature. Despite its ecological significance, few studies have been published, leaving its geographical distribution and functional roles largely unexplored. To address these gaps, extensive surveys are necessary to elucidate the ecological functions and distribution patterns of *Trichoderma* in freshwater environments.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Data availability

All data that support the findings of this study are available in the main text.

REFERENCES

Au DWT, Hodgkiss IJ, Vrijmoed LLP (1992) Fungi and cellulolytic activity associated with decomposition of *Bauhinia purpurea* leaf litter in a polluted and unpolluted Hong Kong waterway. Canadian Journal of Botany 70: 1071–1079. https://doi.org/10.1139/b92-131

Bao DF, Hyde KD, Maharachchikumbura SSN, Perera RH, Thiyagaraja V, Hongsanan S, Wanasinghe DN, Shen H-W, Tian X-G, Yang LQ, Nalumpang S, Luo Z-L (2023) Taxonomy, phylogeny and evolution of freshwater Hypocreomycetidae (Sordariomycetes). Fungal Divers 121 (1): 1–94. https://doi.org/10.1007/s13225-023-00521-8

Bishnoi NR, Kumar R, Bishnoi K (2007) Biosorption of Cr (VI) with *Trichoderma viride* immobilized fungal biomass and cell free Ca-alginate beads. Indian Journal of Experimental Biology 45 (7): 657–64.

Bissett J (1984) A revision of the genus *Trichoderma*. I. Section *Longibrachiatum* sect. nov. Canadian Journal of Botany 62 (5): 924–931. https://doi.org/10.1139/b84-131

Bissett J (1991a) A revision of the genus *Trichoderma*. II. Infrageneric classification. Canadian Journal of Botany 69 (11): 2357–2372. https://doi.org/10.1139/b91-297

Bissett J (1991b) A revision of the genus *Trichoderma*. III. Section *Pachybasium*. Canadian Journal of Botany 69 (11): 2373–2417. https://doi.org/10.1139/b91-298

Brito VN, Lana Alves J, Sírio Araújo K, de Souza Leite T, Borges de Queiroz C, Liparini Pereira O, de Queiroz MV (2023) Endophytic *Trichoderma* species from rubber trees native to the Brazilian Amazon, including four new species. Frontiers in Microbiology 14: 1095199. https://doi.org/10.3389/fmicb.2023.1095199

Bucher VVC, Pointing SB, Hyde KD, Reddy CA (2004) Production of wood decay enzymes, loss of mass, and lignin solubilization in wood by diverse tropical freshwater fungi. Microbial Ecology 48 (3): 331–337. https://doi.org/10.1007/s00248-003-0132-x

Cabanela M V, Jeewon R, Hyde KD (2007) Morphotaxonomy and phylogeny of *Paoayensis lignicola* gen. et sp. nov. (ascomycetes) from submerged wood in Paoay Lake, Ilocos Norte, the Philippines. Cryptogamie Mycologie 28 (4): 301–310.

Cai F, Druzhinina IS (2021) In honor of John Bissett: authoritative guidelines on molecular identification of *Trichoderma*. Fungal Diversity 107 (1): 1–69. https://doi.org/10.1007/s13225-020-00464-4

Calabon M, Bagacay JFE, Nim JA, Sadaba R, Ferriols VME., Concha MJ, Velo J, Jones EBG (2024) *Phaeoacremonium philippinense* (Togniniales, Sordariomycetes), a novel freshwater fungus from the Philippines. Phytotaxa 644 (3): 177–189. https://doi.org/10.11646/phytotaxa.644.3.1

Calabon MS, Hyde KD, Jones EBG, Luo ZL, Dong W, Hurdeal VG, Gentekaki E, Rossi W, Leonardi M, Thiyagaraja V, Lestari AS, Shen HW, Bao DF, Boonyuen N, Zeng M (2022) Freshwater fungal numbers. Fungal Diversity 114 (1): 3–235. https://doi.org/10.1007/s13225-022-00503-2

Calabon MS, Hyde K, Jones E, Bao D, Bhunjun C, Phukhamsakda C, Shen H, Gentekaki E, Al Sharie A, Barros J, Chandrasiri KSU, Hu DM, Hurdeal VG, Rossi W, Valle LG, Zhang H, Figueroa M, Raja HA, Seena S, Song HY, Dong W, El-Elimat T, Leonardi M, Li Y, Li YJ, Luo ZL, Ritter CD, Strongman DB, Wei MJ, Balasuriya A (2023) Freshwater fungal biology. Mycosphere 14 (1):195–413. https://doi.org/10.5943/mycosphere/14/1/4

Cai F, Druzhinina IS (2021) In honor of John Bissett: authoritative guidelines on molecular identification of *Trichoderma*. Fungal Diversity 107: 1–69. https://doi.org/10.1007/s13225-020-00464-4

Cai F, Dou K, Wang P, Chenthamara K, Chen J, Druzhinina IS (2022) The current state of *Trichoderma* taxonomy and species identification. In: Amaresan N, Sankaranarayanan A, Dwivedi MK, Druzhinina IS (Eds.) Advances in *Trichoderma* biology for agricultural applications. Springer, Cham, Switzerland 3–35. https://doi.org/10.1007/978-3-030-91650-3_1

Chaverri P, Branco-Rocha F, Jaklitsch W, Gazis R, Degenkolb T, Samuels GJ (2015) Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. Mycologia 107 (3): 558–590. https://doi.org/10.3852/14-147

Concha MJ, Bagacay JFEV, Calabon MS (2024) Two new records of freshwater Sordariomycetes, *Sporoschisma chiangraiense* (Chaetosphaeriales) and *Trichoderma pinicola* (Hypocreales), from the Philippines. New Zealand Journal of Botany: https://doi.org/10.1080/0028825x.2024.2392911

Cuevas V, Banaay CG (2022) In situ bioremediation and crop growth promotion using *Trichoderma* microbial inoculant (TMI) ameliorate the effects of Cu contamination in lowland rice paddies. Philippine Journal of Science 151 (3): 1255–1265. https://doi.org/10.56899/151.03.37

Cuevas V, Sinohin AM, Orajay JI (2005) Performance of selected Philippine species of *Trichoderma* as biocontrol agents of damping off pathogens and as growth enhancer of vegetables in farmers' field. Philippine Agricultural Scientist 88: 63–71.

Degenkolb T, Dieckmann R, Nielsen KF, Gräfenhan T, Theis C, Zafari D, Chaverri P, Ismaiel A, Brückner H, von Döhren H, Thrane U, Petrini O, Samuels GJ (2008) The *Trichoderma brevicompactum* clade: a separate lineage with new species, new peptaibiotics, and mycotoxins. Mycological Progress 7 (3): 177–219. https://doi.org/10.1007/s11557-008-0563-3

Delsuc F, Brinkmann H, Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. Nature Reviews Genetics 6: 361–375. https://doi.org/10.1038/nrg1603

Dissanayake AJ, Bhunjun CS, Maharachchikumbura SSN, Liu JK (2020) Applied aspects of methods to infer phylogenetic relationships amongst fungi. Mycosphere 11: 2652–2676. https://doi.org/10.5943/mycosphere/11/1/18

Druzhinina IS, Kopchinskiy AG, Kubicek CP (2006) The first 100 *Trichoderma* species characterized by molecular data. Mycoscience 47 (2): 55–64. https://doi.org/10.1007/s10267-006-0279-7

Druzhinina IS, Kopchinskiy AG, Komoń M, Bissett J, Szakacs G, Kubicek CP (2005) An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. Fungal Genetics and Biology 42 (10): 813–828. https://doi.org/10.1016/j.fgb.2005.06.007

Gal-Hemed I, Atanasova L, Komon-Zelazowska M, Druzhinina IS, Viterbo A, Yarden O (2011) Marine isolates of *Trichoderma* spp. as potential halotolerant agents of biological control for arid-zone agriculture. Applied and Environ Microbiology 77 (15): 5100–5109. https://doi.org/10.1128/aem.00541-11

Glez-Peña D, Gómez-Blanco D, Reboiro-Jato M, Fdez-Riverola F, Posada D (2010) ALTER: program-oriented conversion of DNA and protein alignments. Nucleic Acids Research 38: W14–W18. https://doi.org/10.1093/nar/gkq321

Goh TK, Hyde KD (1996) Biodiversity of freshwater fungi. Journal of Industrial Microbiology and Biotechnology 17 (5–6): 28–345. https://doi.org/10.1007/bf01574764

Goh J, Nam B, Lee JS, Mun Hye Yeon, Oh Y, Lee HB, Chung N, Choi Y-J (2018) First report of six *Trichoderma* species isolated from freshwater environment in Korea. The Korean Journal of Mycology 46(3): 213–225. http://doi.org/10.4489/kjm.20180027

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. https://doi.org/citeulike-article-id:691774

Hanada RE, de Jorge Souza T, Pomella AWV, Hebbar KP, Pereira JO, Ismaiel A, Samuels GJ (2008) *Trichoderma martiale* sp. nov., a new endophyte from sapwood of *Theobroma cacao* with a potential for biological control. Mycological Research 112 (11): 1335–1343. https://doi.org/10.1016/j.mycres.2008.06.022

Harman G, Kubicek C (Eds) (2020) Morphology and identification of *Trichoderma*. In: Harman GE, Kubicek CP (Eds.) *Trichoderma* and *Gliocladium*. Volume 1. CRC Press, Boca Raton, Florida, USA, 3–34. https://doi.org/10.1201/9781482295320-8

Hu DM, Liu F, Cai L (2013) Biodiversity of aquatic fungi in China. Mycology 4: 125–168. https://doi.org/10.1080/21501203.2013.835752

Huang J, Wang J, Liu S (2024) Advances in the production of fungi-derived lignocellulolytic enzymes using agricultural wastes. Mycology 15: 523–537. https://doi.org/10.1080/21501203.2023.2253827

Huang S-K, Jeewon R, Hyde KD, Bhat DJ, Wen T-C (2018) Novel taxa within Nectriaceae: *Cosmosporella* gen. nov. and *Aquanectria* sp. nov. from freshwater habitats in China. Cryptogamie, Mycologie 39 (2): 169–192. https://doi.org/10.7872/crym/v39.iss2.2018.169

Hyde KD, Fryar S, Tian Q, Bahkali AH, Xu J (2016) Lignicolous freshwater fungi along a north–south latitudinal gradient in the Asian/Australian region; can we predict the impact of global warming on biodiversity and function? Fungal Ecology 19: 190–200. https://doi.org/10.1016/j.funeco.2015.07.002

Inglis PW, Mello SCM, Martins I, Silva JBT, Macêdo K, Sifuentes DN, Valadares-Inglis MC (2020) *Trichoderma* from Brazilian garlic and onion crop soils and description of two new species: *Trichoderma azevedoi* and *Trichoderma peberdyi*. PLoS ONE 15 (3): e0228485. https://doi.org/10.1371/journal.pone.0228485

Jaklitsch WM, Voglmayr H (2015) Biodiversity of *Trichoderma* (Hypocreaceae in Southern Europe and Macaronesia. Studies in Mycology 80 (1): 1–87. https://doi.org/10.1016/j.simyco.2014.11.001

Jones EBG, Hyde KD, Pang KL (2014) Introduction. In: Jones G, Hyde KD, Pang K-L (Eds.) Freshwater Fungi. Walter de Gruyter, Berlin, Germany, 1–22.

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20: 1160–1166. https://doi.org/10.1093/bib/bbx108

Khan RAA, Najeeb S, Hussain S, Xie B, Li Y (2020a) Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms 8 (6): 817. https://doi.org/10.3390/microorganisms8060817

Khan RAA, Najeeb S, Mao Z, Ling J, Yang Y, Li Y, Xie B (2020b) Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic bacteria and root-knot nematode. Microorganisms 8 (3): 401. https://doi.org/10.3390/microorganisms8030401

Kredics L, Büchner R, Balázs D, Allaga H, Kedves O, Racić G, Varga A, Nagy VD, Vágvölgyi C, Sipos G (2024) Recent advances in the use of *Trichoderma*-containing multicomponent microbial inoculants for pathogen control and plant growth promotion. World Journal of Microbiology and Biotechnology 40: 162. https://doi.org/10.1007/s11274-024-03965-5

Kuhls K, Lieckfeldt E, Samuels GJ, Kovacs W, Meyer W, Petrini O, Gams W, Börner T, Kubicek CP (1996) Molecular evidence that the asexual industrial fungus *Trichoderma reesei* is a clonal derivative of the ascomycete *Hypocrea jecorina*. Proceedings of the National Academy of Sciences of the United States of America 93 (15): 7755–7760. https://doi.org/10.1073/pnas.93.15.7755

Kullnig-Gradinger CM, Szakacs G, Kubicek CP (2002) Phylogeny and evolution of the genus *Trichoderma*: a multigene approach. Mycological Research 106 (7): 757–767. https://doi.org/10.1017/S0953756202006172

Launio CC, Labon KO, Bañez AA, Batani RS (2020) Adoption and economic analysis of using biological control in Philippine highland farms: case of *Trichoderma koningii* strain KA. Crop Protection 136: 105177. https://doi.org/10.1016/j.cropro.2020.105177

Lechat C, Fournier J (2015) *Varicosporella*, a new aquatic genus in the Nectriaceae from France. Ascomycete.org 7 (1): 1–8. https://doi.org/10.25664/art-0121

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16 (12): 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Ma J, Tsegaye E, Li M, Wu B, Jiang X (2020) Biodiversity of *Trichoderma* from grassland and forest ecosystems in northern Xinjiang, China. 3 Biotech 10 (8): 362. https://doi.org/10.1007/s13205-020-02301-6

Marcet-Houben M, Gabaldón T (2009) The tree versus the forest: The fungal tree of life and the topological diversity within the yeast phylome. d'Enfert C (Ed.). PLoS ONE 4: e4357. https://doi.org/10.1371/journal.pone.0004357

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop, GCE 2010, New Orleans, USA, 1–8. https://doi.org/10.1109/gce.2010.5676129

Paz Z, Komon-Zelazowska M, Druzhinina IS, Aveskamp MM, Shnaiderman A, Aluma Y, Carmeli S, Ilan M, Yarden O (2010) Diversity and potential antifungal properties of fungi associated with a Mediterranean sponge. Fungal Diversity 42 (1): 17–26. https://doi.org/10.1007/s13225-010-0020-x

Pearsoon CH (1794) Neuer Versuch einer systematischen Eintheilung der Schwämme. Neues Magazin für die Botanik in ihrem ganzen Umfange 1: 63–80

Pinruan U, Pinnoi A, Hyde KD, Jones EBG (2014) Tropical peat swamp fungi with special reference to palms. In: Jones EBG, Hyde KD, Pang KL (Eds.) Freshwater Fungi. De Gruyter, Berlin, Germany, 371–388. https://doi.org/10.1515/9783110333480.371

Qin W-T, Zhuang W-Y (2016) Seven wood-inhabiting new species of the genus *Trichoderma* (Fungi, Ascomycota) in Viride clade. Scientific Reports 6 (1): 27074. https://doi.org/10.1038/srep27074

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97 (1): 84–98. https://doi.org/10.3852/mycologia.97.1.84

Robbertse B, Reeves JB, Schoch CL, Spatafora JW (2006) A phylogenomic analysis of the Ascomycota. Fungal Genetics and Biology 43: 715–725. https://doi.org/10.1016/j.fgb.2006.05.001

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sadfi-Zouaoui N, Hannachi I, Rouaissi M, Hajlaoui MR, Rubio MB, Monte E, Boudabous A, Hermosa MR (2009) Biodiversity of *Trichoderma* strains in Tunisia. Canadian Journal of Microbiology 55 (2): 154–162. https://doi.org/10.1139/w08-101

Savory JG (1954) Breakdown of timber by Ascomycetes and Fungi Imperfecti. Annals of Applied Biology 41: 336–347. https://doi.org/10.1111/j.1744-7348.1954.tb01126.x

Senanayake IC, Rathnayaka AR, Marasinghe DS, Calabon MS, Gentekaki E, Lee HB, Hurdeal VG, Pem D, Dissanayake LS, Wijesinghe SN, Bundhun D, Nguyen TT, Goonasekara ID, Abeywickrama PD, Bhunjun CS, Jayawardena RS, Wanasinghe DN, Jeewon R, Bhat DJ, Xiang MM (2020) Morphological approaches in studying fungi: collection, examination, isolation, sporulation and preservation. Mycosphere 11 (1): 2678–2754. https://doi.org/10.5943/mycosphere/11/1/20

Siddiquee S (2017) Morphology-based characterization of *Trichoderma* species. In: Practical handbook of the biology and molecular diversity of *Trichoderma* species from tropical regions. Fungal Biology. Springer, Cham, Switzerland, 41-73. https://doi.org/10.1007/978-3-319-64946-7_4

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stielow JB, Lévesque CA, Seifert KA, Meyer W, Irinyi L, Smits D, Renfurm R, Verkley GJM, Groenewald M, Chaduli D, Lomascolo A, Welti S, Lesage-Meessen L, Favel A, Al-Hatmi AMS, Damm U, Yilmaz N, Houbraken J, Lombard L, Quaedvlieg W, Binder M, Vaas LAI, Vu D, Yurkov A, Begerow D, Roehl O, Guerreiro M, Fonseca A, Samerpitak K, van Diepeningen AD, Dolatabadi S, Moreno LF, Casaregola S, Mallet S, Jacques N, Roscini L, Egidi E, Bizet C, Garcia-Hermoso D, Martín MP, Deng S, Groenewald JZ, Boekhout T, de Beer ZW, Barnes I, Duong TA, Wingfield MJ, de Hoog GS, Crous PW, Lewis CT, Hambleton S, Moussa TAA, Al-Zahrani HS, Almaghrabi OA, Louis-Seize G, Assabgui R, McCormick W, Omer G, Dukik K, Cardinali G, Eberhardt U, de Vries M, Robert V (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia - Molecular Phylogeny and Evolution of Fungi 35: 242–263. https://doi.org/10.3767/003158515X689135

Subramaniam S, Zainudin NAIM, Aris A, Hasan ZAE (2022) Role of *Trichoderma* in plant growth promotion. In: Amaresan N, Sankaranarayanan A, Dwivedi MK, Druzhinina IS (Eds.) Advances in *Trichoderma* Biology for Agricultural Applications. Springer, Cham, Switzerland, 257–280. https://doi.org/10.1007/978-3-030-91650-3_9

Tang GT, Li Y, Zhou Y, Zhu YH, Zheng XJ, Chang XL, Zhang S-R, Gong G-S (2022) Diversity of *Trichoderma* species associated with soil in the Zoige alpine wetland of Southwest China. Scientific Reports 12 (1): 21709. https://doi.org/10.1038/s41598-022-25223-0

Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Ściseł J (2022) *Trichoderma*: the current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. International Journal of Molecular Sciences 23 (4): 2329. https://doi.org/10.3390/ijms23042329

White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds.) PCR protocols, a guide to methods and applications. Academic Press, San Diego, USA, 315–322.

Wijayawardene NN, Hyde KD, Al-Ani LKT, Tedersoo L, Haelewaters D, Rajeshkumar KC, Zhao RL, Aptroot A, Leontyev DV, Saxena RK, Tokarev YS, Dai DQ, Letcher PM, Stephenson SL, Ertz D, Lumbsch HT, Kukwa M, Issi IV, Madrid H, Phillips AJL, Selbmann L, Pfliegler WP, Horváth E, Bensch K, Kirk PM, Kolaříková K, Raja HA, Radek R, Papp V, Dima B, Ma J, Malosso E, Takamatsu S, Rambold G, Gannibal PB, Triebel D, Gautam AK, Avasthi S, Suetrong S, Timdal E, Fryar SC, Delgado G, Réblová M, Doilom M, Dolatabadi S, Pawłowska JZ, Humber RA, Kodsueb R, Sánchez-Castro I, Goto BT, Silva DKA, de Souza FA, Oehl F, da Silva GA, Silva IR, Błaszkowski J, Jobim K, Maia LC, Barbosa FR, Fiuza PO, Divakar PK, Shenoy BD, Castañeda-Ruiz RF, Somrithipol S, Lateef AA, Karunarathna SC, Tibpromma S, Mortimer PE, Wanasinghe DN, Phookamsak R, Xu J, Wang Y, Tian F, Alvarado P, Li DW, Kušan I, Matočec N, Mešić A, Tkalčec Z, Maharachchikumbura SSN, Papizadeh M, Heredia G, Wartchow F, Bakhshi M, Boehm E, Youssef N, Hustad VP, Lawrey JD, Santiago ALCMA, Bezerra JDP, Souza-Motta CM, Firmino AL, Tian Q, Houbraken J, Hongsanan S, Tanaka K, Dissanayake AJ, Monteiro JS, Grossart HP, Suija A, Weerakoon G, Etayo J, Tsurykau A, Vázquez V, Mungai P, Damm U, Li QR, Zhang H, Boonmee S, Lu YZ, Becerra AG, Kendrick B, Brearley FQ, Motiejūnaitė J, Sharma B, Khare R, Gaikwad S, Wijesundara DSA, Tang LZ, He MQ, Flakus A, Rodriguez-Flakus P, Zhurbenko MP, McKenzie EHC, Stadler M, Bhat DJ, Liu JK, Raza M, Jeewon R, Nassonova ES, Prieto M, Jayalal RGU, Erdoğdu M, Yurkov A, Schnittler M, Shchepin ON, Novozhilov YK, Silva-Filho AGS, Gentekaki E, Liu P, Cavender JC, Kang Y, Mohammad S, Zhang LF, Xu RF, Li YM, Dayarathne MC, Ekanayaka AH, Wen TC, Deng CY, Pereira OL, Navathe S, Hawksworth DL, Fan XL, Dissanayake LS, Kuhnert E, Grossart HP, Thines M (2020) Outline of Fungi and fungus-like taxa. Mycosphere 11: 1160–1456. https://doi.org/10.5943/mycosphere/11/1/8

Wong MKM, Goh T-K, Hodgkiss IJ, Hyde KD, Ranghoo VM, Tsui CKM, Ho W-H, Wong WSW, Yuen T-K (1998) Role of fungi in freshwater ecosystems. Biodiversity and Conservation 7 (9): 1187–1206. https://doi.org/10.1023/a:1008883716975

Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J (2023) *Trichoderma* and its role in biological control of plant fungal and nematode disease. Frontiers in Microbiology 14: https://doi.org/10.3389/fmicb.2023.1160551

Ye C, Jing T, Sha Y, Mo M, Yu Z (2023) Two new *Trichoderma* species (Hypocreales, Hypocreaceae) isolated from decaying tubers of *Gastrodia elate*. MycoKeys 99: 187–207. https://doi.org/10.3897/mycokeys.99.109404

Zafra G, Cortés-Espinosa D V (2015) Biodegradation of polycyclic aromatic hydrocarbons by *Trichoderma* species: a mini review. Environmental Science and Pollution Research 22 (24): 19426–19433. https://doi.org/10.1007/s11356-015-5602-4

Zeng Z-Q, Zhuang W-Y (2022) New species of Nectriaceae (Hypocreales) from China. Journal of Fungi 8(10): 1075. https://doi.org/10.3390/jof8101075

Zhang H, Jones GEB, Zhou D, Bahkalf AH, Hyde KD (2011) Checklist of freshwater fungi in Thailand. Cryptogamie, Mycologie 32: 199–217. https://doi.org/10.7872/crym.v32.iss2.2011.199

Zhao R, Mao L-J, Zhang C-L (2023) Three new species of *Trichoderma* (Hypocreales, Hypocreaceae) from soils in China. MycoKeys 97: 21–40. https://doi.org/10.3897/mycokeys.97.101635

Zhao R, Chen K-Y, Mao L-J, Zhang C-L (2024) Eleven new species of *Trichoderma* (Hypocreaceae, Hypocreales) from China. Mycology, 1–30. https://doi.org/10.1080/21501203.2024.2330400

Zheng H, Qiao M, Lv Y, Du X, Zhang KQ, Yu Z (2021) New species of *Trichoderma* isolated as endophytes and saprobes from Southwest China. Journal of Fungi 7 (6): 467. https://doi.org/10.3390/jof7060467

Zhu ZX, Zhuang WY (2015) Three new species of *Trichoderma* with hyaline ascospores from China. Mycologia 107 (2): 328–345. https://doi.org/10.3852/14-141

Zin NA, Badaluddin NA (2020) Biological functions of *Trichoderma* spp. for agriculture applications. Annals of Agricultural Sciences 65 (2): 168–178. https://doi.org/10.1016/j.aoas.2020.09.003